ASSOCIATION OF HLA-DRB1 ALLELES AND BASAL CELL CARCINOMA (BCC) OF THE SKIN IN BASRAH

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Abstract
Immunogenetic factors have been explored as being a possible risk factor in the aetiology of basal cell carcinoma (BCC). This study aimed to clarify the association of HLA-DRB1 alleles in immunocompetent patients with BCC in Basrah city. A total of 27 patients with confirmed BCC and 31 controls were analyzed for HLA-DRB1 genotyping using generic SSP2LB one lambda micro ssp- HLA-DNA typing tray.
Results showed a positive association of BCC with HLA DRB1*07 (p-value 0.0280, OR=3.7143, 95% CI=1.1521-11.9743), HLA alleles of DRB1*11 (OR=2.2), DRB1*04 (OR=1.4), DRB1*01 (OR=1.125) have a higher frequency among cases but the association is not significant. These finding suggest a possible immunogenetic role in the development of BCC.

Introduction
Skin cancer has a major share among total cancer cases in Basra, with basal cell carcinoma (BCC) as predominant type. In Iraq it ranks nine for the most common cancer. UV radiation is considered the main risk factor. Fair skin, older age, male sex, smoking, systemic Immunosuppression and prior radiation therapy are additional risk factors.
The DRB1 is a protein coding gene that belongs to class II beta chain, together with alpha chain (DRA) forming class II heterodimer that plays a central role in the immune system by presenting processed peptides derived from extra cellular protein on the antigen presenting cells (APC).
Association of Non melanoma skin cancer (NMSC) with alleles of the human leucocyte antigen (HLA) have been reported with both class I and class II alleles but the exact mechanism is not clear. In the immunocompetent population, BCC was positively associated with HLA-DR1, DR7 and HLA-DR7. However conflicting studies also available by Rompel 1995 who concluded that there is a negative association with multiple BCC and also by Emtestam 1996 in Swedish population, While in transplant patients negative association was concluded by Glove (1993) and Ingvar (2012).
This study aimed to investigate the association between BCC and the presence of HLA–DRB1 alleles especially DRB1*07, DRB1*04, and DRB1*01 in Basrah, Southern Iraq.

Materials and methods
A case control study was conducted in Al-Sadir Teaching Hospital from the period November 2013 to May 2015 including 27 cases of histologically confirmed basal cell carcinoma of the skin and 30 controls of similar age that were randomly selected. DNA was extracted from blood samples for all the cases and controls using Blood Mini DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. HLA-DRB1 genotyping was done using generic SSP2LB one lambda micro SSP HLA DNA typing tray (one lambda) according to the manufacturer’s instruction and the PCR product were analyzed with 2.5% agarose gel electrophoresis. The typing results was
interpreted using the assistance of the HLA software available from One Lambda, Inc. (HLA Fusion program).

**Statistical analysis**

Data analysis was performed using (SPSS) for Windows (version 15). Pearson chi-square was used to determine any significant difference in the distribution of genotypes frequency between cases and controls and Fisher exact test was used if the cell counts less than 5. The association between the genotype and risk of BCC were evaluated by computing the odds ratios (OR) and their 95% confidence intervals (CI). Probability levels less than 0.05 were used as a criterion of significance.

**Results**

Twelve distinct HLA–DRB1 alleles were identified in 27 patients and 30 controls. DRB1-07 appears to be a risk factor for BCC, the odds ratio (OR) = 3.7 and the 95% confidence interval (1.15-11.9) and the p value is 0.028. There was no statistical significance association between the presence of DRB1*11 (OR=2.2), DRB1*04 (OR=1.4) or DRB1*01 (OR=1.25). Other frequent alleles were DRB1*03, DRB1*13, DRB1*15 in both cases and controls group while DRB1*09 and DRB1*12 were absent in both groups. DRB1*10 and DRB1*14 showed higher frequency in controls than cases group (Table I).

<table>
<thead>
<tr>
<th>DRB1* allele groups</th>
<th>Patients N=27(%)</th>
<th>Controls N=30(%)</th>
<th>P value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>3 (11.1)</td>
<td>3 (10)</td>
<td>0.8915</td>
<td>1.125</td>
<td>0.207-6.11</td>
</tr>
<tr>
<td>03</td>
<td>6 (22.2)</td>
<td>8 (26.6)</td>
<td>0.697</td>
<td>0.7857</td>
<td>0.233-26500</td>
</tr>
<tr>
<td>04</td>
<td>7 (25.9)</td>
<td>6 (20)</td>
<td>0.5953</td>
<td>1.4000</td>
<td>0.4045-4.8449</td>
</tr>
<tr>
<td>07</td>
<td>13 (40.7)</td>
<td>6 (20)</td>
<td>0.0280</td>
<td>3.7143</td>
<td>1.1521-11.9743</td>
</tr>
<tr>
<td>08</td>
<td>1 (3.7)</td>
<td>1 (3.33)</td>
<td>0.9395</td>
<td>1.1154</td>
<td>0.0664-18.7492</td>
</tr>
<tr>
<td>09</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.9591</td>
<td>1.1091</td>
<td>0.0213-57.8168</td>
</tr>
<tr>
<td>10</td>
<td>1 (3.7)</td>
<td>5 (16.6)</td>
<td>0.1448</td>
<td>0.1923</td>
<td>0.0210-1.7638</td>
</tr>
<tr>
<td>11</td>
<td>12 (37.03)</td>
<td>8 (26.6)</td>
<td>0.1637</td>
<td>2.2000</td>
<td>0.7254-6.6725</td>
</tr>
<tr>
<td>12</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.9591</td>
<td>1.1091</td>
<td>0.0213-57.8168</td>
</tr>
<tr>
<td>13</td>
<td>3 (11.1)</td>
<td>4 (13.3)</td>
<td>0.7988</td>
<td>0.8125</td>
<td>0.1646-4.0102</td>
</tr>
<tr>
<td>14</td>
<td>0 (0)</td>
<td>4 (13.3)</td>
<td>0.1404</td>
<td>0.1071</td>
<td>0.0055-2.0872</td>
</tr>
<tr>
<td>15</td>
<td>7 (25.9)</td>
<td>9 (30)</td>
<td>0.7327</td>
<td>0.8167</td>
<td>0.2555-2.6108</td>
</tr>
</tbody>
</table>

**Table I: Gene frequency distribution of DRB*1 allele among cases and controls.**

HLA- DRB1 typing electrophoresis results

**Figure 1:** 2.5% agarose gel electrophoresis photo of one sample showing results of DRB1 typing, the internal control band is amplified in all loaded wells except the negative control well (1H), positive bands are noted in the wells 1B, 1D, 3B, 3D, 3H.

![Image of electrophoresis result](image-url)
Discussion

Although the sequence based typing (SBT) is considered the gold standard for resolution of HLA typing, the PCR-SSP (Sequence Specific Primers) method is an accurate, highly sensitive and fast that results can be achieved within two hours and is ideal for HLA –DR typing. This study showed that DRB1*07 is associated significantly with increased risk of BCC and confirmed to some extent the results of other studies by Elshazly in Egyptian (2010) and by Bavinck (2000) on the tropical Island of Saba and in renal transplant IS patients (2002) indicating that DRB1*07 is associated with significant increased risk to BCC.

In the present study besides DRB1*07, the frequencies of DR11 in the BCC group was higher than those of the control group, although the P values of this allele was not significant (DR11 OR=2.2) which could be attributed to the small sample size. This allele was negatively associated with BCC in Egyptian study (Elshazly 2010). In contrast with Elshazly finding of DRB1*01 positive association in Egyptian population, the frequency of DRB1*01 did not reach significant level in our study (OR=1.25). This positive association also proposed by Myskowski et al. 1985 and de carvalho et al. 2012 in renal transplant patients in southern Brazil.

A possible explanation for the apparent conflicting associations between HLA-DRB1*11, DRB1*01 and the development of BCC may be attributed to different genetic background of the studied populations.

In conclusion the results of this study confirmed that DRB1*07 is associated with BCC and support the hypothesis that immune system might have a role in BCC aetiology.

References